

What is claimed is:

1. A method of determining amino acid sequence of a polypeptide, comprising:

- (a) constructing a graph from mass spectra of
5 two or more differentially labeled polypeptides, said graph comprising a node with mass m , number of labels n , intensity i , and mass differential of labels δ ;
(b) creating a node corresponding to a paired signal having masses of about m and about $m+n\delta$, and
10 (c) adding a labeled weighted directed edge to said graph between any two nodes corresponding to a mass of an amino acid, said labeled weighted directed edge combining properties of said paired signals.

2. The method of claim 1, further
15 comprising:

- (a) creating a source node with total mass M , total number of labels N and fixed intensity I_s ; and
(b) creating a terminus node with mass 0, minimum number of labels n_0 , and fixed intensity I_t ;

20 3. The method of claim 2, further comprising selecting a path from the source node to the terminus node.

25 4. The method of claim 3, further comprising computing a priority score for each path through the graph.

5. The method of claim 1, wherein said differential label marks an internal amino acid residue.

6. The method of claim 1, wherein said differential label marks a terminal amino acid residue.

7. The method of claim 1, wherein said differential label marks a terminal and an internal amino acid residue.

8. The method of claim 1, wherein said differentially labeled polypeptides further comprise stable isotopic labels.

9. The method of claim 1, wherein said differentially labeled polypeptides further comprise heavy and light labeled isotopes selected from the group consisting of hydrogen, carbon, oxygen, nitrogen, sulfur and selenium.

10. The method of claim 1, wherein said differentially labeled polypeptides further comprise an unlabeled polypeptide and a labeled polypeptide.

11. The method of claim 1, wherein said polypeptide is labeled *in vivo* or *in vitro*.

12. The method of claim 1, wherein said mass spectra are obtained from a mass spectrometry database.

13. The method of claim 1, wherein said mass spectra are of low resolution.

14. The method of claim 1, further comprising masses of amino acid post-translational modifications.

15. The method of claim 1, further comprising adding complement node with mass $M-m$, and a number of labels $N-n+n_0$.

16. The method of claim 1, further comprising
5 including multiple amino acid edges between nodes, said multiple amino acid edges characterizing a degenerate amino acid residue in said polypeptide sequence.

17. The method of claim 1, wherein steps a-c are repeated one or more times.

10 18. The method of claim 1, wherein steps a-c are performed by an automated process.

19. A method of determining an amino acid sequence of a polypeptide, comprising:

15 (a) differentially labeling two or more polypeptide mixtures, and
(b) determining an amino acid sequence of a polypeptide within said mixture using the method of claim 1.

20 20. The method of claim 19, wherein said differential label marks an internal amino acid residue.

21. The method of claim 19, wherein said differential label marks a terminal amino acid residue.

22. The method of claim 19, wherein said
25 differential label marks a terminal and an internal amino acid residue.

23. The method of claim 19, wherein said differentially labeled polypeptides further comprise stable isotopic labels.

24. The method of claim 19, wherein said 5 differentially labeled polypeptides further comprise heavy and light labeled isotopes selected from the group consisting of hydrogen, carbon, oxygen, nitrogen, sulfur and selenium.

25. The method of claim 19, wherein said 10 differentially labeled polypeptides further comprise an unlabeled polypeptide and a labeled polypeptide.

26. The method of claim 19, wherein said polypeptide is labeled *in vivo* or *in vitro*.

27. The method of claim 19, wherein said mass 15 spectra are obtained from a mass spectrometry database.

28. The method of claim 19, wherein said mass spectra are of low resolution.

29. The method of claim 19, further comprising separating components of said mixture.

20 30. A method of determining an amino acid sequence of a parent polypeptide, comprising:

(a) obtaining mass spectra of two or more differentially labeled polypeptide fragments of a parent polypeptide;

- (b) assigning a mass and a weighting characteristic to two or more paired signals having a difference in mass corresponding to an integer value of said differential label, said weighting characteristic 5 combining properties of each signal within said paired signals;
- (c) selecting from said mass spectra a paired signal having said assigned mass and a weighting characteristic distinguishable from non-peptide signals, 10 said assigned mass indicating the mass of a polypeptide fragment within said spectra;
- (d) determining the difference in mass of said polypeptide fragments;
- (e) assigning said mass differences a 15 satisfying amino acid name, and
- (f) orienting said assigned amino acid names.

31. The method of claim 30, wherein said differential label marks an internal amino acid residue.

32. The method of claim 30, wherein said 20 differential label marks a terminal amino acid residue.

33. The method of claim 30, wherein said differential label marks a terminal and an internal amino acid residue.

34. The method of claim 30, wherein said 25 differentially labeled polypeptides further comprise stable isotopic labels.

35. The method of claim 30, wherein said differentially labeled polypeptides further comprise heavy and light labeled isotopes selected from the group consisting of hydrogen, carbon, oxygen, nitrogen, sulfur 5 and selenium.

36. The method of claim 30, wherein said differentially labeled polypeptides further comprise an unlabeled polypeptide and a labeled polypeptide.

37. The method of claim 30, wherein said 10 parent polypeptide is labeled *in vivo* or *in vitro*.

38. The method of claim 30, wherein said mass spectra are obtained from a mass spectrometry database.

39. The method of claim 30, wherein said mass spectra are of low resolution.

15 40. A method of determining an amino acid sequence of a parent polypeptide, comprising:

(a) obtaining a mass spectra of two differentially labeled polypeptide fragments of said parent polypeptide, said differential label marking a 20 terminal residue and at least one internal amino acid residue;

(b) identifying a paired signal from said mass spectra corresponding to an internal amino acid residue, said paired amino acid signal having a 25 difference in mass corresponding to said differential label;

(c) identifying a paired signal from said mass spectra corresponding to said terminal residue, said

paired amino acid signal having a difference in mass corresponding to said differential label;

(d) determining the difference in mass of polypeptide fragments corresponding to said identified
5 paired signals;

(e) assigning said mass differences a satisfying amino acid name, and

(f) orienting said assigned amino acid names.

41. The method of claim 40, wherein said
10 differential label marks two or more internal amino acid residues.

42. The method of claim 40, wherein said differential label marks two terminal amino acid residues.

15 43. The method of claim 40, wherein said differential label marks a terminal and two or more internal amino acid residues.

44. The method of claim 40, wherein said differentially labeled polypeptides further comprise a
20 stable isotopic label.

45. The method of claim 40, wherein said differentially labeled polypeptides further comprise heavy and light labeled isotopes selected from the group consisting of hydrogen, carbon, oxygen, nitrogen, sulfur
25 and selenium.

46. The method of claim 40, wherein said differentially labeled polypeptides further comprise an unlabeled polypeptide and a labeled polypeptide.

47. The method of claim 40, wherein said parent polypeptide is labeled *in vivo* or *in vitro*.

48. The method of claim 40, wherein said mass spectra are obtained from a mass spectrometry database.

5 49. The method of claim 40, wherein said mass spectra are of low resolution.

50. The method of claim 40, further comprising identifying a paired signal corresponding to a different internal residue having an integer difference
10 in mass corresponding to said differential label.

51. The method of claim 40, further comprising identifying a paired signal corresponding to two or more internal amino acid residues having the same integer difference in mass.

15 52. The method of claim 40, wherein said step of orienting said assigned names further comprises assigning a weighted value to said paired amino acid signals.

20 53. The method of claim 40, wherein said terminal residue comprises the lowest integer difference in mass.

54. The method of claim 40 wherein said terminal residue is a carboxyl terminus.

25 55. The method of claim 40, wherein said terminal residue is an amino terminus.